Bis(Dpa-ZnII) Appended Xanthone: Excitation Ratiometric Chemosensor for Phosphate Anion Derivatives Based on Anion-Induced Rearrangement of Coordination Chemistry

Akio Ojida, Hiroshi Nonaka, Yoshifumi Miyahara, Shun-ichi Tamaru, Kazuki Sada, and Itaru Hamachi*

Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura Campus, Nishikyo-ku, Kyoto, 615-8510, Japan

Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka, 819-0395, Japan

PRESTO (Synthesis and Control, JST)
Scheme S1. Synthesis of the chemosensor 1-2Zn\textsuperscript{II}; (a) AcONa, H\textsubscript{2}O, reflux (b) pivaloyl chloride, NEt\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C (c) NBS, BPO, CCl\textsubscript{4}, reflux (d) 2,2’-dipicolylamine, K\textsubscript{2}CO\textsubscript{3}, DMF, rt (e) NaOH, THF-MeOH, 0 °C (f) Zn(NO\textsubscript{3})\textsubscript{2}, MeOH, rt

Synthesis of the chemosensor 1-2Zn\textsuperscript{II} (Scheme S1)
3,6-Dihydroxy-1,8-dimethyl-xanthone (5) \textsuperscript{[10]}
A mixture of 4 (1.70 g, 6.20 mmol) and sodium acetate (5.09 g, 62.0 mmol) in H\textsubscript{2}O (40 mL) was refluxed for 26 h. After cooling to 0 °C, the pH of the suspension was adjusted to 4 ~5 with 1N HCl, and the precipitate was collected by filtration. The solid was washed with H\textsubscript{2}O and dried in vacuo to give 5 (1.49 g, 94%) as a pale yellow powder.

\textsuperscript{1}H-NMR (400 MHz, DMSO) δ 2.69 (6H, s), 6.56 (2H, d, J=2.4 Hz), 6.59 (2H, d, J=2.4 Hz). FAB-MS m/e 257.1 [M+H]\textsuperscript{+}.

1,8-Dimethyl-3,6-dipivaloxyxanthone (6)
To a cooled (0 °C) solution of 5 (700 mg, 2.73 mmol), triethylamine (1.14 mL, 8.19 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (20 mL) was added dropwise pivaloyl chloride (0.74 mL, 6.00 mmol). The reaction mixture was stirred at 0 °C for 10 min and further stirred for 20 min at room temperature. After addition of water, the mixture was extracted with ethyl acetate (x2), and the combined organic layers were washed with saturated NaHCO\textsubscript{3} and brine followed by drying over MgSO\textsubscript{4}. The solvent was evaporated, and the residue was filtered and washed with hexane to give 6 (963 mg, 83%) as a colorless powder.
\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{)} \delta 1.38 (18H, s), 2.88 (6H, s), 6.82 (2H, d, \text{J}=2.0 \text{ Hz}), 7.03 (2H, d, \text{J}=2.0 \text{ Hz}). \text{FAB-MS m/e} 425.3 \text{ [M+H]}^+. \]

**1,8-Bis[(2,2'-dipicolylamino)methyl]-3,6-dipivaloxyxanthone (7)**

A degassed solution of 6 (600 mg, 1.41 mmol), N-bromosuccinimide (NBS) (552 mg, 3.10 mmol), and a catalytic amount of benzoyl peroxide (BPO) in CCl\(_4\) (40 mL) was refluxed for 4 h, during which time a catalytic amount of BPO was added at each 30 min, and the reaction was monitored by \(^1\text{H-NMR}. \) After cooling to room temperature, the solvent was evaporated, and the residue was dissolved in AcOEt. The organic layer was washed with saturated NaHCO\(_3\) and brine followed by drying over MgSO\(_4\). After removal of the solvent in vacuo, the solid was collected by filtration and washed with hexane/i-Pr\(_2\)O (1 : 1) to give 1,8-dibromo-3,6-dipivaloxyxanthone (532 mg) as a mixture with the corresponding tribromo compound. The ratio of the dibromo and tribromo compound was determined to be ca. 2 : 1 by \(^1\text{H-NMR}. \) FAB-MS m/e 583.1 [M+H]\(^+\) (dibromo compound) and 661.0 [M+H]\(^+\) (tribromo compound).

To a cooled (0 °C) solution of 2,2'-dipicolylamine (357 mg, 1.79 mmol) and K\(_2\)CO\(_3\) (370 mg) in anhydrous DMF (10 mL) was added 1,8-dibromo-3,6-dipivaloxyxanthone (520 mg, a mixture with the tribromo compound), and the solution was stirred for 1 h at rt. After dilution with H\(_2\)O, the mixture was extracted with AcOEt (x2), and the combined organic layers were washed with H\(_2\)O and brine followed by drying over MgSO\(_4\). The solvent was removed in vacuo, and the residue was purified by flash column chromatography on silica gel (CH\(_2\)Cl\(_2\) : MeOH : aqueous NH\(_3\) = 300 : 10 : 1 \rightarrow 200 : 10 : 1). The obtained solid was washed with i-Pr\(_2\)O to give 7 (369 mg, 33% from 6) as a pale yellow powder. \(^1\text{H-NMR (400 MHz, CDCl}_3\text{)} \delta 1.38 (18H, s), 3.98 (8H, s), 4.51 (4H, s), 7.07 (2H, d, \text{J}=2.4 \text{ Hz}), 7.09-7.12 (4H, m), 7.53 (4H, d, \text{J}=7.6 \text{ Hz}), 7.59 (4H, dt, \text{J}=2.0, 7.6 \text{ Hz}), 7.83 (2H, d, \text{J}=2.4 \text{ Hz}), 8.51 (4H, dd, \text{J}=0.8, 4.8 \text{ Hz}). \text{FAB-MS m/e} 819.5 [M+H]\(^+\).

**1,8-Bis[(2,2'-dipicolylamino)methyl]-3,6-dihydroxy-xanthone (1)**

A solution of 7 (150 mg, 0.18 mmol) and 4N NaOH (1 mL) in THF-MeOH (2 : 1, 6 mL) was stirred for 90 min at 0 °C. After neutralization with 1N HCl, the solution was extracted with CH\(_2\)Cl\(_2\) containing a small amount of MeOH (x3). The combined
organic layers were concentrated in vacuo, and the residue was filtered and washed with 
CH₂Cl₂/hexane (1 : 1) and H₂O to give 1 (104 mg, 87%) as a colorless powder.

¹H-NMR (400 MHz, CD₃OD) δ 3.93 (4H, s), 4.41 (8H, s), 6.57 (2H, d, J=2.4 Hz), 7.24 (4H, t, J=5.6 Hz), 7.32 (2H, m), 7.67 (4H, t, J=7.6 Hz), 7.74 (4H, t, J=7.6 Hz), 8.40 (4H, d, J=7.6 Hz). ¹³C-NMR (150 MHz, DMSO) δ 56.60, 60.01, 100.24, 112.16, 113.12, 122.15, 122.19, 136.67, 144.67, 148.92, 157.82, 159.15, 162.07, 177.88. FAB-MS m/e 651.30 [M+H]+. FAB-HRMS m/e calcld for [M+H]+ 651.2720, found 651.2719. Anal Calcd for C₃₉H₃₄N₆O₄·H₂O C: 70.04, H: 5.43, N: 12.57. Found C:70.50, H: 5.16, N: 12.60.

3,6-Dihydroxy-1,8-bis[(2,2'-dipicolylamino)methyl]xanthone 2Zn(NO₃)₂ complex (1·2Zn²⁺)

Aqueous solution of Zn(NO₃)₂ (304 mM; 211 µL, 0.062 mmol) was added to a solution of 1 (20.9 mg, 0.032 mmol) in distilled MeOH (5 mL), and the mixture was stirred for 20 min at room temperature. After removal of the solvent in vacuo, the residue was dissolved in distilled H₂O, and the solution was filtered through cellulose acetate filter (pore size, 0.45 µm) and lyophilized. The obtained solid was filtered and washed with AcOEt to give 1·2Zn²⁺ (30.5 mg, 92%) as a pale yellow powder.

¹H-NMR (400 MHz, CD₃OD) δ 4.04 (4H, dd, J=15.2 Hz ), 4.23 (4H, dd, J=16.0 Hz ), 4.49 (4H, s), 6.68 (2H, d, J=2.4 Hz), 6.90 (2H, d, J=2.4 Hz), 7.27 (4H, d, J=8.0 Hz), 7.47 (4H, t, J=6.0 Hz), 7.91 (4H, m), 8.66 (4H, m). ¹³C-NMR (150 MHz, DMSO) δ 57.21, 60.03, 102.44, 113.63, 121.15, 124.16, 124.76, 136.35, 140.79, 147.21, 154.52, 158.05, 163.13, 181.46. FAB-MS m/e 903.0 [M-2NO₃]⁺. Anal Calcd for C₃₉H₃₄N₆O₄·2Zn(NO₃)₂ C: 45.50, H: 3.33, N: 13.61. Found C:45.31, H: 3.35, N: 13.48.
Scheme S2. Synthesis of the chemosensor 2-Zn\textsuperscript{II}; (a) NBS, BPO, CCl\textsubscript{4}, reflux (b) 2,2’-dipicolylamine, K\textsubscript{2}CO\textsubscript{3}, DMF, rt (c) NaOH, THF-MeOH, 0 °C (f) Zn(NO\textsubscript{3})\textsubscript{2}, MeOH, rt.

Synthesis of the chemosensor 2-Zn\textsuperscript{II} (Scheme S2)

1-Methyl-8-[(2,2’-dipicolylamino)methyl]-3,6-Dipivaloxyxanthone (8)

This compound was synthesized by the same procedure described for the synthesis of 7. The bromination of 6 (1.00 g, 2.36 mmol) with 2.2 equiv of NBS gave the monobromo compound as a mixture with the corresponding dibromo compound (monobromo : dibromo = ca. 0.7 : 1), which was subsequently reacted with 2,2’-dipicolylamine and purified with flash column chromatography (CH\textsubscript{2}Cl\textsubscript{2} : MeOH : aqueous NH\textsubscript{3} = 300 : 10 : 1 → 250 : 10 : 1) to give 8 (375 mg, 26% from 6) as a pale yellow powder.

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) δ 1.35 (9H, s), 1.39 (9H, s), 2.83 (3H, s), 4.01 (4H, s), 4.57 (2H, s), 6.79 (1H, d, J=2.4 Hz), 7.03 (1H, d, J=2.0 Hz), 7.07 (1H, d, J=2.4 Hz), 7.10 (2H, m), 7.56 (2H, d, J=7.6 Hz), 7.10 (2H, dt, J=1.2, 4.0 Hz), 7.87 (1H, d, J= 2.4Hz), 8.52 (2H, d, J=4.0 Hz). FAB-MS m/e 622.3 [M+H]\textsuperscript{+}.

3,6-Dihydroxy 8-methyl-1-[(2,2’-Dipicolylamino)methyl]xanthone (2)

This compound was synthesized from 8 (210 mg, 0.34 mmol) by the same procedure described for the synthesis of 1. Yield 91% (139 mg).

\textsuperscript{1}H-NMR (400 MHz, DMSO) δ 2.67 (3H, s), 3.63 (4H, s), 4.34 (2H, s), 6.57 (1H, s) , 6.62 (1H, s) , 6.66 (1H, s) , 7.25-7.28 (2H, m) , 7.47 (1H, s) 7.62 (2H, d, J=7.2 Hz) , 7.79 (2H, t, J=7.6 Hz) , 8.49 (2H, d, J=4.0Hz), 10.63 (1H, s), 10.76 (1H, s). FAB-MS m/e 454.2 [M+H]\textsuperscript{+}. FAB-HRMS m/e calcld for [M+H]\textsuperscript{+} 454.1767, found 454.1768.

3,6-Dihydroxy-8-methyl-1-[(2,2’-Dipicolylamino)methyl]xanthone Zn(NO\textsubscript{3})\textsubscript{2} complex (2-Zn\textsuperscript{II})
This compound was prepared from 2 (30.3 mg, 0.068 mmol) by complexation with 1 equiv of Zn(NO$_3$)$_2$ as described for the synthesis of 1-2Zn$^{II}$. Yield 80% (34.5 mg).

$^1$H-NMR (400 MHz, CD$_3$OD) δ 2.91 (3H, s), 4.09 (2H, s), 4.39 (2H, d, J=16.0 Hz), 4.98 (2H, d, J=15.6 Hz), 6.53 (1H, d, J=2.0 Hz), 6.56 (1H, d, J=2.4 Hz), 6.67-6.68 (1H, m), 6.98 (1H, d, J=2.4 Hz), 7.37 (2H, m), 7.45 (2H, m), 7.94 (2H, t, J=7.2 Hz), 8.72 (2H, m).

FAB-MS m/e 579.1 [M-NO$_3$]$^+$. FAB-HRMS m/e calcd for [M-NO$_3$]$^+$ 579.0858, found 579.0861. Anal Calcd for C$_{27}$H$_{23}$N$_5$O$_{10}$·2H$_2$O: C: 47.77, H: 4.01, N: 10.32. Found C: 48.15, H: 3.64, N: 9.99.

**Fluorescence Study**

Fluorescence spectra were recorded on a Perkin-Elmer LS55 spectrometer. The fluorescence quantum yield of 1-2Zn$^{II}$ was determined under neutral aqueous conditions (50 mM HEPES, 50 mM NaCl, pH 7.2), by using quinine sulfate in 0.1 N H$_2$SO$_4$ as a standard. The titration experiments with the phosphate anion species (Figure 3, Table 1) were carried out with a solution (3 mL) of 1-2Zn$^{II}$ or 2-Zn$^{II}$ (3 ~10 µM) in 50 mM HEPES, 50 mM NaCl, pH 7.2 in a quartz cell at 20 °C. The fluorescence excitation spectral change (emission wavelength $\lambda_{em}$ = 480 nm) was monitored upon the addition of the freshly prepared aqueous solution of the phosphate species with a micro syringe. Fluorescent titration curves at 407 nm were analyzed with the nonlinear least-square curve-fitting method or Benesi-Hildebrand plot to evaluate the apparent binding constant ($K_{app}$, M$^{-1}$).
ITC measurement

ITC titration was performed on an Isothermal Titration Calorimeter from MicroCal Inc. The measurement was conducted at 298 K. A solution of ATP (0.5 mM) in 50 mM HEPES, 50 mM NaCl (pH 7.2) was injected stepwise (5 µL x 56 times) to a solution of 1-2Zn\(^{II}\) (25 µM) dissolved in the same solvent system. The measured heat flow was recorded as function of time and converted into enthalpies (\(\Delta H\)) by integration of the appropriate reaction peaks. Dilution effects were corrected by subtracting the results of a blank experiment with a solution of 50 mM HEPES, 50 mM NaCl (pH 7.2) in place of the ATP solution under the identical experimental conditions. The binding parameters (\(K_{app}, \Delta H, \Delta S, n\)) were evaluated by applying one site model using the software Origin (MicroCal Inc.).

**Figure S1.** ITC titration curve and processed date for the titration of ATP with 1-2Zn\(^{II}\). Measurement conditions: [1-2Zn\(^{II}\)] = 25 µM, [ATP] = 0.5 mM (5 µL x 56 injections), 50mM HEPES, 50mM NaCl, pH 7.2 ,25 °C.